

August 11, 1993, now abandoned, which is a continuation of 07/881,607, filed May 12, 1992, now abandoned, which is a continuation of U.S.S.N. 07/368,674, filed June 19, 1989, now abandoned, which is a continuation-in-part of U.S.S.N. 06/887,070, filed July 17, 1986, now abandoned."

IN THE CLAIMS:

Please cancel claims 5, 9 and 10 without prejudice or disclaimer of the subject matter contained therein.

Please amend the following claims as indicated:

3. (Amended) A method of identifying a functional [DNA] nucleotide sequence which provides a desired biological activity comprising:
- a. providing a means for detecting said desired biological activity;
  - b. synthesizing a mixed population of random [DNA] nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;
  - c. introducing a plurality of the random [DNA] nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing random [DNA] nucleotide sequences;
  - d. introducing said cloning vectors into suitable host cells;
  - e. expressing said cloning vectors in said host cells; and
  - f. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors

which comprise a functional [DNA] nucleotide sequence which provides the desired biological activity.

4. (Amended) A method of isolating a functional [DNA] nucleotide sequence which provides a desired biological activity comprising:
  - a. providing a means for detecting said desired biological activity;
  - b. synthesizing a mixed population of random [DNA] nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;
  - c. introducing a plurality of said random [DNA] nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing random [DNA] nucleotide sequences;
  - d. introducing said cloning vectors into suitable host cells;
  - e. expressing said cloning vectors in said host cells;
  - f. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors which comprise a functional [DNA] nucleotide sequence which provides the desired biological activity; and
  - g. isolating said [polynucleotide] nucleotide sequence or sequences which provide the desired biological activity.

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6. (Amended) A method of isolating a host [cells] cell which comprises a functional [DNA] nucleotide sequence which produces a desired biological activity comprising:

- a. providing a means for detecting said desired biological activity;
- b. synthesizing a mixed population of random oligonucleotides by enzymatic or chemical synthesis without regard to a wild type sequence;
- c. introducing a plurality of said random oligonucleotides into a population of cloning vectors to obtain a plurality of cloning vectors containing random oligonucleotides;
- d. introducing said cloning vectors into suitable host cells;
- e. expressing said cloning vectors in said host cells;
- f. screening said host cells to determine whether the inserted oligonucleotide provides the desired biological activity;
- g. isolating said host cells having said oligonucleotide having the desired biological activity.

7. (Amended) A method of producing a mixed population of random [DNA] nucleotide sequences in order to identify one or more functional sequences which provide a desired biological activity comprising:

- a. synthesizing a mixed population of random [DNA] nucleotide sequences in a manner by which the frequency of stop codons in said mixed population is reduced; and
- b. inserting said mixed population of random [DNA] nucleotide sequences into

a population of cloning vectors to form a mixed population of vectors containing randomly generated sequences.

8. (Amended) An isolated, mixed population of vectors comprising randomly generated [DNA] nucleotide sequences encoding a mixed population of amino acid sequences and having a reduced frequency of stop codons as compared to codons encoding [other] amino acids.

11. (Amended) An isolated, mixed population of random [DNA] nucleotide sequences comprising a [DNA] nucleotide sequence providing a desired biological activity produced by a method comprising synthesizing a mixed population of random [DNA] nucleotide sequences in a manner which [results in stop codon bias] biases against stop codons, and introducing a plurality of said randomly generated [DNA] nucleotide sequences into a population of cloning vectors to form a mixed population of vectors containing randomly generated [DNA] nucleotide sequences.

12. (Amended) A method of identifying a functional [DNA] nucleotide sequence which provides a desired biological activity comprising:

- a. providing a means for detecting said desired biological activity;
- b. synthesizing a mixed population of random [DNA] nucleotide sequences in a

manner by which the frequency of stop codons in said mixed population is reduced;

c. introducing a plurality of random [DNA] nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing random [DNA] nucleotide sequences;

d. introducing said cloning vectors into suitable host cells;

e. expressing said cloning vectors in said host cells; and

f. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors which comprise a functional [DNA] nucleotide sequence which provides the desired biological activity.

13. (Amended) A method of identifying a peptide, polypeptide or protein having a desired biological activity comprising:

a. providing a means for detecting said desired biological activity;

b. synthesizing a mixed population of random [DNA] nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;

c. introducing a plurality of said random [DNA] nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing random [DNA] nucleotide sequences;

d. introducing said cloning vectors into suitable host cells;

e. expressing said cloning vectors in said host cells to produce a random population of peptides, polypeptides or proteins; and

f. screening said random population of peptides, polypeptides or proteins with said means for detecting the desired biological activity under conditions which allow detection of one or more [random population of] peptides, polypeptides or proteins from said random population having the desired biological activity.

14. (Amended) A method of identifying a peptide, polypeptide or protein capable of [binding a preselected antigen] reacting with a substrate:

a. providing [an antigen] a substrate;

b. synthesizing a mixed population of random [DNA] nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;

c. introducing a plurality of said random [DNA] nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing random [DNA] nucleotide sequences;

d. introducing said cloning vectors into suitable host cells;

e. expressing said cloning vectors in said host cells to produce a random population of peptides, polypeptides or proteins; and

f. screening said random population of peptides, polypeptides or proteins with said [preselected antigen] substrate under conditions which allow detection of one or more

[random population of] peptides, polypeptides or proteins from said random population which  
[bind to the antigen] react with said substrate.

Please add new claims 15-25 as follows:

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15. A process for the production of a peptide or protein having a desired biological activity comprising the steps of:

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producing by enzymatic or chemical synthesis a random population of nucleotide sequences without regard to a wild type sequence;

forming a library of expression vectors containing the random population of nucleotide sequences;

culturing host cells containing the vectors to produce peptides or proteins encoded by the random population of nucleotide sequences;

carrying out screening or selection on the host cells, to identify a peptide or protein produced by the host cells having the desired biological function;

isolating a randomly synthesized nucleotide sequence which encodes the identified peptide or protein; and

using the isolated sequence to produce the peptide or protein having the desired biological activity.

16. A method of identifying a peptide or protein having a desired biological activity,

comprising:

- (a) producing a population of peptides or proteins encoded by random nucleotide sequences produced by enzymatic or chemical synthesis without regard to a wild type sequence; and
- (b) screening said population of peptides or proteins for said desired biological activity under conditions which allow detection of one or more peptides or proteins having said desired biological activity.

17. A method of producing a peptide or protein having a desired biological function, comprising:

- (a) producing a population of peptides or proteins encoded by random nucleotide sequences produced by enzymatic or chemical synthesis without regard to a wild type sequence;
- (b) screening said population of peptides or proteins for said desired biological function under conditions which allow detection of one or more peptides, polypeptides or proteins having said desired biological function;
- (c) isolating the nucleotide sequence(s) encoding said one or more peptides or proteins having said desired biological property; and
- (d) producing said peptide or protein.



18. A method of producing a random polynucleotide population for use in screening for a desired biological function, comprising adding random nucleotides to an expression vector without regard to a wild type sequence.

19. A method of generating a product of an enzyme-substrate reaction, comprising combining a population of peptides or proteins encoded by random nucleotide sequences produced without regard to a wild type sequence with substrate under conditions such that said enzyme-substrate reaction may occur, and incubating said population of peptides or proteins with said substrate such that said product may be detected.

20. A method of identifying a population of peptides or proteins which catalyze an enzyme substrate reaction, comprising:

(a) combining a population of peptides or proteins encoded by random nucleotide sequences produced without regard to a wild type sequence with substrate under conditions such that said enzyme-substrate reaction may occur;

(b) incubating said population of peptides or proteins with said enzyme substrate so that a product of said enzyme-substrate reaction may be generated; and

(c) screening for the product of the enzyme-substrate reaction to identify a population of peptides or proteins which catalyze said enzyme-substrate reaction.

21. A process for the production of an expression vector capable of transcribing or translating an open reading frame to produce a desired biological function, said vector comprising a random nucleotide sequence, comprising the steps of:

producing a random population of nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;

ligating said random population of nucleotide sequences into an expression vector to form a library of expression vectors;

transforming suitable host cells with said library of expression vectors;

growing the transformed host cells containing said expression vectors;

screening said transformed host cells in order to identify an expression vector capable of transcribing or translating an open reading frame to produce the desired biological function, or selecting said host cells containing an expression vector capable of transcribing or translating an open reading frame to produce the desired biological function;

isolating the identified or selected transformed host cell; and

isolating the expression vector from said isolated host cell.

22. A method for producing a random population of vectors comprising:

(a) synthesizing a heterogenous population of random nucleotide sequences comprising about a billion different nucleotide sequences, said method consisting of random ligation of oligonucleotides or random addition of nucleotide triphosphates without regard to a wild type

sequence, and

(b) inserting said heterogenous population of random nucleotide sequences into a population of vectors to form a heterogenous population of vectors containing random nucleotide sequences.

23. A process for the production of a nucleotide sequence comprising,  
producing a heterogenous population of random nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;  
inserting said population of random nucleotide sequences into vectors to form a random population of vectors;  
introducing said random population of vectors into host cells in a manner to produce a random population of transformed host cells;  
growing independent colonies from the transformed host cells;  
screening and/or selecting said colonies of the host cells to identify host cells comprising a nucleotide sequence having a desired biological activity; and  
isolating said nucleotide sequence from the selected or screened host cells.

24. A method of identifying a nucleotide sequence having a desired biological activity, comprising:

(a) producing a population of nucleotide sequences comprising greater about a billion

different random nucleotide sequences by enzymatic or chemical synthesis without regard to a wild type sequence;

(b) screening said population of nucleotide sequences for said desired biological activity under conditions which allow detection of nucleotide sequences having said desired biological activity.

25. A method of identifying a functional nucleotide sequence which provides a desired biological activity comprising:

- a. providing a means for detecting said desired biological activity;
- b. forming a population of cloning vectors, each containing a random nucleotide sequence produced by enzymatic or chemical synthesis without regard to a wild type sequence;
- c. introducing said cloning vectors into suitable host cells;
- d. expressing said cloning vectors in said host cells; and
- e. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors which comprise a functional nucleotide sequence which provides the desired biological activity. --